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THE BIOLOGICAL TESTING OF AERIAL SPRAY DEPOSITS

1948 Season

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P R O G R E S S R E P O R T

THE BIOLOGICAL TESTING OF AERIAL SPRAY DEPOSITS
1948 Season

SUMMARY

Studies of airplane spray deposits carried on at this station during 1946 and 1947 were continued through 1948 using nymphs of the American roach Periplaneta americana L. as test animals in biological evaluation of the spray deposits. Both rearing and testing methods are reviewed with the inclusion of various changes made through three years experience.

The laboratory spray chamber was used for small scale simulated aerial deposits. Modifications in the chamber and its use are discussed.

Laboratory tests using roach nymphs show:

a. Fuel oil in standard spray solution can cause roach mortality until the spray deposit is dry.

b. Fine drop spray deposits on foliage cause higher initial kill but coarse drop deposits have longer residual effect. The same effect is obtained with deposits on glass.

c. DDT deposits appear to cause higher mortality among male than among female roach nymphs but further investigation is needed.

d. Storage temperatures between -2°C and 38°C do not alter the biological effectiveness of New York State formulation A-2.

A series of photographs for use in visual estimation of airplane spray deposits is described.

I N T R O D U C T I O N

Studies of the factors affecting airplane application of insecticides for forest insect control were begun at this station in 1946 and continued in 1947 and 1948. The greater part of this work has been in field tests performed jointly by the laboratory staff and described elsewhere. Techniques used in these biological investigations have been described previously (Eaton and Secrest, 1947). New techniques and changes in established procedures with an account of various laboratory experiments performed to study spray deposits are described here. The work was performed by the writers with the assistance, for periods indicated, of R. W. Hartmeyer (Jan.-March), B. K. Dozier (June 7-Sept. 27), R. S. Andres (June 14-Sept. 10) D. Buchanan (Sept. 30-Oct. 22) and M. W. Statler (Oct. 13 through Dec.).

OBJECTIVES

1. Maintenance of the stock of test insects in large enough quantities to fill all needs of field and laboratory experiments.

2. Biological testing of airplane spray deposits.

3. Development of equipment for simulating aerial sprays in the laboratory.

4. Studies of the factors affecting the action of residual sprays on the test insect.

METHODS

It had previously been determined that the american roach Periplaneta americana L. is best suited as a test insect for biologically testing aerial spray deposits 1/ thus this insect was used exclusively during 1948. While rearing and testing methods have been described before 1/ 2/ they will be reviewed again to include details of some changes made through three years experience.

Rearing

Cultures of P. americana are maintained at a level to provide all necessary 3 week old nymphs for field and laboratory tests. Rearing is done in a special rearing room equipped with an electric forced draft thermostatically controlled heating unit which directs warm air over an 18 x 22 inch tray filled with water and containing a number of 6 inch flower pots. This provides heat and humidity control to maintain a temperature of about 80°F. and a relative humidity of about 45%. Windows are kept covered with paper to prevent direct rays of sunlight from getting to the roaches as it has been found to cause some mortality. Sunlight must also be kept off petri dishes during biological tests for the same reason.

Cultures are maintained in 9 x 12 cm. glass battery jars and provided with a strip of 1/4-inch mesh hardware cloth about 3 x 18 inches bent in a loop and indented on top to support a 1 inch shell vial. Food is given in the form of dog biscuits and water is kept in the shell vial plugged with wet cotton. When newly hatched nymphs are first placed in the jars, water is provided from an 8 oz. glass jar inverted on a pad of wet cotton in a 3-1/2 inch petri dish. This is left undisturbed until depleted (about 4 weeks) and has apparently been the means of eliminating occasional cases of high mortality in jars of young nymphs. Figure 1 and Figure 2. All other jars of roaches are fed and watered weekly or oftener if necessary.

After the last moult, adult roaches are removed from the nymphs and placed in clean jars with a one inch layer of sawdust on the bottom for the burial of egg cases.

At intervals of one to two weeks adults are removed from their jars and the sawdust sifted through a coarse screen (1/8 inch mesh) to separate the egg cases. The egg cases are then placed in covered petri dishes to await hatching. The petri dishes are kept on a tray supported by tripods standing in water to maintain humidity requirements and to discourage the depredations of ants and the escape of nymphs. It has been found that in cold weather when the room temperature is not constant a 60 watt electric light kept burning beneath the tray of petri dishes helps to maintain the production rate. When moving roaches from one jar

Figure 1.--Equipment for cultures of newly hatched roach nymphs.

Figure 2.--Equipment for cultures of older nymphs and adults.

to another most workers prefer to etherize them for easier handling. When this is done a cotton pad with ether (diethyl ether) is placed in the jar and the jar covered until the roaches become sluggish but not completely inactivated. Over etherization has been found to cause egg mortality before the dosage is high enough to cause any permanent injury to the adults.

Each day or every other day until all eggs are hatched nymphs are removed from dishes containing egg cases. This is done by sifting with a piece of 1/3 inch mesh screen over the dish and shaking the dish over a jar until the nymphs have all fallen through. By this method a rapid and complete separation is accomplished without damage to the eggs or nymphs. Figure 3.

Figure 3.--Cultures of egg cases with screen used for separating nymphs from eggs.

The floor and shelves of the rearing room are kept clean of dust and debris and care is taken not to provide harbors for escaped roaches. Food is kept in covered metal containers. Occasional scrubbing of the floor with water containing a pine tar oil reduces the odor associated with roaches. Sodium flouride powder sprinkled along the base of the walls and across the door sill provides a fatal poison dose to escaping roaches without endangering the stock. It has been found that by using the methods described here one worker can adequately care for the rearing room in 15 to 20 hours each week.

Figure 4.--View of one side of rearing room.

Figure 5.--Rearing room showing temperature and humidity control apparatus, tray for dishes of egg cases and metal food cans.

Biological Testing.

Biological tests of airplane spray deposits collected in the field test flights at Beltsville were made much the same as in previous years. The lower sections of petri dishes were exposed beneath the plane. Ten minutes after release of spray over the dishes they were covered, numbered and collected. In the laboratory these dishes were laid out in sets according to flight number, a clean dish added to each set as a control and 15 three week old roach nymphs placed in each dish. These tests were set up in the laboratory at any convenient time within three days after the flight as it has been determined that there is no change in the ultimate result when covered dishes are held over for this period. To provide food and water and thus prevent canabalism a small pellet made from pulverized dog biscuit moistened with 50% glycerine in distilled water is provided in each dish. Figure 6. The glycerine prevents mold

Figure 6.--Test dish set up with roach nymphs and food pellet.

formation during the summer months. Biological effectiveness is determined by the mortality of the roaches after three days on the treated dishes (six days when dishes have been exposed to weathering). Roaches are considered as dead when they are unable to control their movements, usually evidenced by being on their backs and not able to right themselves as these individuals never recover.

The biological method of testing DDT deposits is also used on foliage treated either in the field or in the laboratory. Roaches are confined in crystalizing dishes, 5 inches in diameter and 2-1/4 inches deep, with treated foliage (usually pine tips) and covered with glass plates 6 x 6 inches or are confined on growing branches in sleeve cages, Figure 7.

Figure 7.--Sleeve cages used to confine roach nymphs on growing branches.

LABORATORY SPRAY TESTING EQUIPMENT

An apparatus for simulating aerial sprays in the laboratory was described by Eaton and Secrest 1/. This apparatus was used for several tests during 1948 and was also modified as seemed necessary. A glass sided enclosure was added to one end to eliminate the towel covering and vacuum cleaner exhaust formerly needed to keep spray from escaping into the room, an exhaust fan with a shutter was installed at the opposite end of the chamber. Also a hand operated shutter control for long exposures was installed. The same fixed nozzle was used in most tests for uniformity and another similar one but adjustable was constructed and used for production of low dosages with fine drops. With this nozzle it is possible to deposit drops of 20 microns mmd with a range of 5 to 75 microns and low enough volume that over one minute exposure is necessary to obtain a deposit of one gallon per acre.

In operation the apparatus is calibrated to the desired dosage each time it is used rather than by means of a calibration curve. This is quickly accomplished by means of a Spencer colorimeter and more accurate dosages are obtained. Drop size may also be determined at each test either by microscopic measurement or photographically as described by Davis 2/. For either method microscope slides coated with "Dri-film" are used to collect the deposit.

LABORATORY INVESTIGATIONS

Effect of Oil Upon Roach Mortality.

In order to determine what effect the oil has upon roach mortality in petri dish tests a series of dishes were sprayed with solutions at the rate of 0.2 gallons per acre in coarse drops and in fine drops. Four different solutions were used containing Sovacide 544-B (1.0 ml.), Dupont Oil Red (9.8 gm.) and fuel oil (enough to make 2000 ml. of spray) with DDT contents of 0, 0.01, 0.1 and 1.0 grams respectively. Deposits at these dosages were tested when fresh, when partially dried and when completely dried. Results are shown in the following tabulation:

Interval Between Spraying & Infestation	Number of Tests	Mortality Percent by Quantity of DDT and Drop Size Class							
		0.0 #/A		0.002 #/A		0.02 #/A		0.2 #/A	
		Coarse	Fine	Coarse	Fine	Coarse	Fine	Coarse	Fine
1/2 day	10 each	82.0	--	82.4*	--	98.7	--	100.0	--
3 days	"	7.3	10.0	12.0	17.3	74.7	82.0	94.7	100.0
7 days	"	0.0	3.3	2.7	5.2	60.7	20.0	100.0	100.0

* 20 Tests in this instance.

These data indicate that the oil alone in the standard spray formula can cause considerable mortality among young roach nymphs when they are confined on freshly sprayed petri dishes. After drying there is practically no effect from the carrier and mortality increases in proportion to the amount of DDT present.

Toxicity of Treated Foliage After Weathering.

Experiments to determine the length of effectiveness of coarse and fine deposits on foliage started in 1947 were continued in 1948. Coarse and fine drop deposits at the rate of 0.15 pounds per 0.15 gallons per acre were made on tips of Pinus virginiana Mill. and at intervals of 1 week were infested with three week old roach nymphs. In the meantime all tips were kept in vials of water in the laboratory. Mortalities after six days exposure on the twigs were as follows:

Interval Between Spraying and Infestation	Number of Tests	Mortality Percent	
		Coarse Drops	Fine Drops
None	10	83.3	93.3
6 days	10	35.3	45.3
10 days	10	39.3	15.3
14 days	10	20.0	5.3

These data are in concurrence with previous data where it was concluded that fine drops cause higher initial mortality but that coarse drop deposits are effective for a longer period of time.

Comparison of Weathering on Coarse and Fine Drop Deposits.

As a beginning in the studies of factors involved in the residual effectiveness of DDT sprays a series of tests were begun to compare coarse drops with fine drops on petri dishes when exposed to the weather, Figure 8. For this purpose 50 petri dishes were sprayed with standard

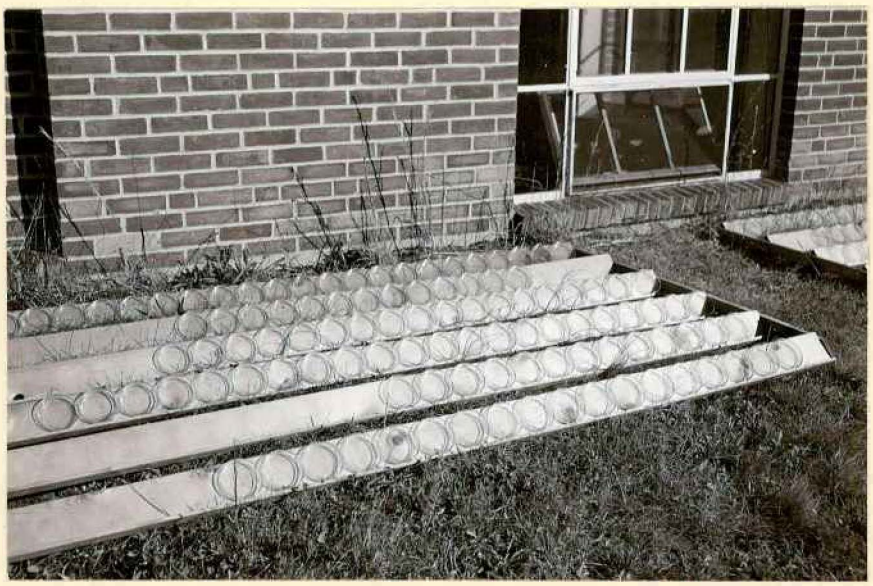

Figure 8.--Method of exposing petri dishes to the weather.

solution an average dosage of 0.55 pounds per acre in fine drops and 0.62 pounds per acre in coarse drops. (Fine drops were 26 microns mmd and coarse drops 203 microns). Ten dishes each were infested with roach nymphs weekly with the following results:

Exposure to Weather	Coarse Drops: No. of Plates	Coarse Drops: Mortality Percent	Fine Drops: No. of Plates	Fine Drops: Mortality Percent	Check Mortality: No. of Plates	Check Mortality: Weathered Percent	Temp. Mean Av. Deg. F.	Rain During Week Inches
None	10	100.0	10	100.0	-	-	-	-
1 week	10	74.7	10	79.3	2	13.3	41.1	3.28
2 weeks	10	66.0	10	72.0	2	0.0	41.8	1.55
3 weeks	10	64.0	10	58.7	2	16.7	38.6	.02
4 weeks	9	81.5	10	74.7	2	6.7	34.5	2.17

NOTE: No mortality in untreated checks except 6.7% at 4th week test.

Comparisons of Weathering on Deposits from Emulsion and Oil Solutions.

A similar series was begun to compare emulsions with standard spray solution. Coarse drops only (300 microns) were used at dosages of 0.98 pounds per acre of emulsion and 0.86 pounds per acre of oil solution. The unweathered series was omitted also. Results were as follows:

Exposure to Weather	Emulsion: No. of Plates	Emulsion: Mortality Percent	Solution: No. of Plates	Solution: Mortality Percent	Check Mortality: No. of Plates	Check Mortality: Weathered Percent	Temp. Mean Av. Deg. F.	Rain During Week Inches
1 week	10	58.7	10	99.3	2	0	38.6	1.54
2 weeks	10	61.3	10	100.0	2	6.7	33.5	.65
3 weeks	10	98.7	10	100.0	2	0	28.9	1.33
4 weeks	(plates blown away by high winds)							

NOTE: No mortality in untreated checks except 3.3% at first week test.

With the limited amount of data on hand conclusions are not yet possible. These tests are being repeated and expanded to include deposits on foliage.

Relation of Sex of Roach Nymphs to Susceptability to DDT.

In view of observed differences in susceptability to DDT of male and female flies and mosquitoes it was decided to ivestigate the possibility of such differences in the nymphs of P. americana. Characteristics for differentiation of 3 week old nymphs were obtained from Mr. A. B. Gurney, Div. Insect Identification and consisted of prescence (female) or absence (male) of a cleft in the posterior border of the subgenital plate (9th segment) as seen from the ventral aspect.

The procedure was to spray a coarse drop deposit on eight petri dish sections using the spray chamber and standard solution. A 6 x 6 inch glass plate was sprayed before the dishes, one after 4 dishes and one after 8 dishes. These deposits were used for colorometric analysis for DDT. The deposit in each case was approximately 0.1 pound per acre. These sprayed dishes plus two untreated controls were each infested with 15 three week old nymphs. At various intervals dead or dying roaches were removed to alcohol for preservation. Results were as follows:

SUSCEPTABILITY OF MALE AND FEMALE ROACH NYMPHS TO DDT DEPOSITS

Experiment Number	Number of Roaches Used: Male	Female	Total	Mortality after 72 hours: Male Percent	Female Percent
1	61	51	112	59.0	49.0
2	66	50	116	73.8	68.0
3	64	56	120	85.9	85.7
4	90	90	130	96.7	96.7
Checks	56	47	103	0.0	0.0

These limited tests show higher mortality among the males than among the females but further tests are needed to prove whether this difference is consistent or whether it is great enough to affect the results of biological testing.

Biological Test of New York State Formulation A-2.

At the request of the Gypsy Moth Laboratories a biological test was made on two samples of New York State formulation A-2 to determine what if any variation might be expected after storage at low temperature. One sample had been held at -2°C and the other at 38°C. Tests were made using P. americana and the usual test procedure with deposits of 0.25 and 0.10 pound per acre applied with the spray chamber. Tests with equal dosages of standard solution were made for comparison. Results showed no significant differences.

VISUAL AIDS TO ESTIMATION OF SPRAY DEPOSITS IN THE FIELD

A series of natural size photographs of spray deposits on "Dri-film" coated glass were prepared. This included various dosages of coarse drops, fine drops and a combination of coarse and fine drops ranging from 0.10 pound per acre to 2.0 pounds per acre. It was intended that these be used as the basis of visual estimation of deposit in the field. Various members of the laboratory staff tested the method by estimating deposit on a series of plates sprayed by airplane in the field and subsequently analyzed for deposit colorometrically with a photometer. Although a few radically different estimates were made the results were close enough that the method could be used in the field for quick estimations.

References

1/ Eaton, C. B. and J. P. Secrest, 1948. Progress Report, The Biological Testing of Aerial Spray Deposits, 1947 Season.

2/ Eaton, C. B., 1947. The Biological Testing of Aerial Spray Deposits, 1946 Season.

3/ Davis, J. M. A Photographic Method for Recording Spray Drops. (unpublished)